CLINICAL STUDY

Study of the dermatophytes in dogs and the risk of human infection

Kozak M, Bilek J, Beladicova V, Beladicova K, Baranova Z, Bugarsky A

The Ist Internal Clinic, University of Veterinary Medicine, Kosice, Slovakia. **kozak@uvm.sk**

Abstract

Background: **The infiltrative growth of fungi that multiplies in the tissues (dermatophytes, candida, agents of visceral mycoses) is the generally known proof of "active" functioning of these organisms. The relative importance of fungi as inducers of human and animal diseases grows constantly.**
Methods: **The study was carried out on dogs that showed skin changes. The frequency of examinations was determined on the basis of anamneses. Skin scrapings and affected fragile or dull hair and skin swabs were subject to Laboratory diagnostic examinations based on cultivation and microscopical examination. Our evaluations were focused on the type of parasite – ecothrix, endothrix, neoendothrix, and the size of arthrospores.**
Results: **The results of our study are presented as the incidence of dermatophytes and other micromycetes in 100 samples of skin scrapings and swabs from 100 dogs of both sexes, different breeds and age categories that had exhibited skin problems. We isolated 12 species. *Malassezia pachydermatis* was the most frequently detected species and was isolated from 31 cases. *Trichophyton mentagrophytes* was isolated from 2 samples, species *Microsporum canis* was isolated only from one case, *Candida albicans* was isolated from 3 samples. The agent *Candida krusei* was isolated from 5 samples, *Candida pulcherrima* a relatively rare candidal species, was isolated from skin of an 8-year old Dalmatian bitch, *Candida sp.* in 5 cases. *Trichosporon cutaneum* was isolated from a 3-year old Laika etc.**
Conclusion: **This study confirms the need of constant research in this area. This indicates that the incidence of mycoses in dogs and other pets is of importance in the exposure of people to the risk of acquiring mycotic infections. *(Tab. 1, Fig. 5, Ref. 57.)***
Key words: appearance, dermatophytes, micromycetes, dog, skin, risk of infection.

When setting the basic goal of our study we considered the real situation in veterinary practice dealing with small animals, and particularly the needs of clinical practice. We focused on the group of dermatophytic and micromycotic agents and their "share" in skin diseases of dogs, the incidence of which is very high in current veterinary practice. We point in parallel to the specificities of their occurrence, course of diseases and specific diagnoses.

Mycotic diseases in animals are either manifested by typical disease changes or persist asymptomatically without evident clinical signs. A too late recognition or too discrete symptoms of untreated animal mycosis can lead to the development of human infections. Working and breeding relations between humans and animals can expose humans to the risk of infection.

Material and methods

The study was carried out at the Ist Internal clinic of the University of Veterinary Medicine (UVM) in Košice that had shown skin changes. Altogether 100 dogs were examined clinically and samples were taken from them for additional examina-

The Ist Internal Clinic, University of Veterinary Medicine, Kosice, State Veterinary Institute, Bratislava, and Skin Clinic, Faculty of Medicine, Safarikiensis University, Kosice, Slovakia

Address for correspondence: M. Kozak, MVD, PhD, The Ist Internal Clinic, University of Veterinary Medicine, Komenskeho 73, SK-041 81 Kosice, Slovakia.
Phone: +421.55.6339410, Fax: +421.55.6323666

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tion. The clinical examination and sampling took place in the period from May 2000 to May 2002. The frequency of examination was determined on the basis of anamnesis. Skin scrapings and affected fragile or dull hair and skin swabs were used for cultivation and microscopical examination.

Laboratory diagnostic examinations were carried out at the State Veterinary Institute in Bratislava, Department of mycology and parasitology that operates as the centre of mycological diagnostics in the Slovak Republic. All samples were examined also at the department of bacteriology.

A sufficient quantity of affected material was sampled from the marginal parts of lesions as spontaneous healing may have occurred in the central regions thus impeding the diagnosis. In addition to the desquamated parts of skin or scabs, the sample had to contain also hair particles. The probability of recovering the agent is decreased considerably by previous treatment or by the use of disinfectants since the primocultures from the treated foci do not have to grow or may provide atypical results that make their identification more difficult.

Each sample was examined *microscopically and by cultivation.* Samples of skin scales, crusts, and hair were placed into 2–3 drops of 10 % KOH, covered with a coverslip and heated midly above the flame (3 times). After 5 to 10 min they were diagnosed microscopically. In addition to that, ink preparations (Parker) were prepared. They were evaluated after 24 hours. Stable preparations were also prepared using either soluble glass or nail polish as solidification of soluble glass takes several hours.

Our evaluations were focused on assessing the type of parasite – ecothrix, endothrix, neoendothrix, and the size of arthrospores.

Samples were cultivated in a conventional way. The scrapings were placed into a Petri dish. Hair and scabs were broken to small pieces (3–4 mm). Using a bacteriological loop, three stabs were performed per one tube. One piece of scab or 2–3 hair pieces were cultivated in one place. Each sample was inoculated into 9 tubes and cultivated on Sabouraud agar enriched by yeast extract, thiamine, antibiotics (streptomycin, chloramphenicol), and actidion (cycloheximide produced by UpJohn Company and Calbiochem Corporation, La Jolla, California). The latter compound serves to suppress the saprophytic species of microscopical fungi the growth of which makes it impossible to isolate the agent. The saprophytic microflora grows more rapidly than the dermatomycotic agents so without actidion it would be impossible to isolate dermatophytes from animal material. Agar slants were inoculated and incubated in a thermostat at 34 °C for 10—20 days. Dermatophytes were cultivated in the media dispensed into tubes because cultivation on Petri dishes is associated with rapid drying and difficult isolation.

Swabs were examined only by cultivation. They were transported from Košice in a transport medium *Fungi quick* (producer Dispolab Žilina), a new sampling system with modified Sabouraud agar. It serves as a transport diagnostic medium of all agents of mycotic infections and can also be used for cultivation of yeast micro-organisms. The proof of fungi and differentiation of individual candida species were carried out after re-inoculation into suitable media.

The inoculated media were examined starting from day 3 up to the establishing of diagnosis that was carried out on day 10 to 12 on average. Microscopical preparations of isolated cultures were prepared using Lugol's solution. The typisation of cultures positive for filamentous fungi was conducted according to their macroscopical and microscopical characteristics, shape, dimensions and arrangement of macroconidia and other parts of isolated cultures.

The cultures that were contaminated were purified by repeated re-inoculation by material from marginal parts of colonies into fresh media. After obtaining pure cultures, physiological identification tests – fermentation and assimilation of saccharides by yeast micro-organisms – were carried out.

Thin layers of Sabouraud medium were also used to identify cultures of yeast microorganisms. In these layers they form pseudomycelium that is characteristic as for individual species and as such it serves as a determination aid.

Results

The results of our study are presented as follows: The incidence of dermatophytes and other micromycetes in 100 samples of skin scrapings and swabs from 100 dogs of both sexes, of different breeds and age categories that exhibited skin problems (Tab. 1, Fig. 1)

Malassezia pachydermatis (Weidman) Dodge

Out of all isolated agents, *Malassezia pachydermatis* was the most frequently detected species and was isolated from 31 cases.

The cultures of this species were cultivated on modified Sabouraud agar with antibiotics and began to grow on days 3 to 4, and in some isolates as late as on day 6.

The colonies of this agent were small, dark-cream to light-brown with greyish surface and easy-to-disturb consistency. Microscopical examination showed spherical and elliptical shapes (Fig. 2)

They multiplied by budding at the wide basis, did not ferment maltose, saccharose, glucose, lactose, galactose, and rafinose. They assimilated maltose and glucose. No hyphae were observed.

Tab. 1. Dermatomycological findings.

Species	Number of positive findings
Malassezia pachydermatis	31
Candida sp.	5
Candida krusei	5
Candida albicans	3
Dematiaceae	3
Trichophyton mentagrophytes	2
Scopulariopsis brevicaulis	2
Microsporum canis	1
Candida pulcherrima	1
Trichosporum cutaneum	1
Geotrichum candidum	1
Chrysosporium pannorum	1

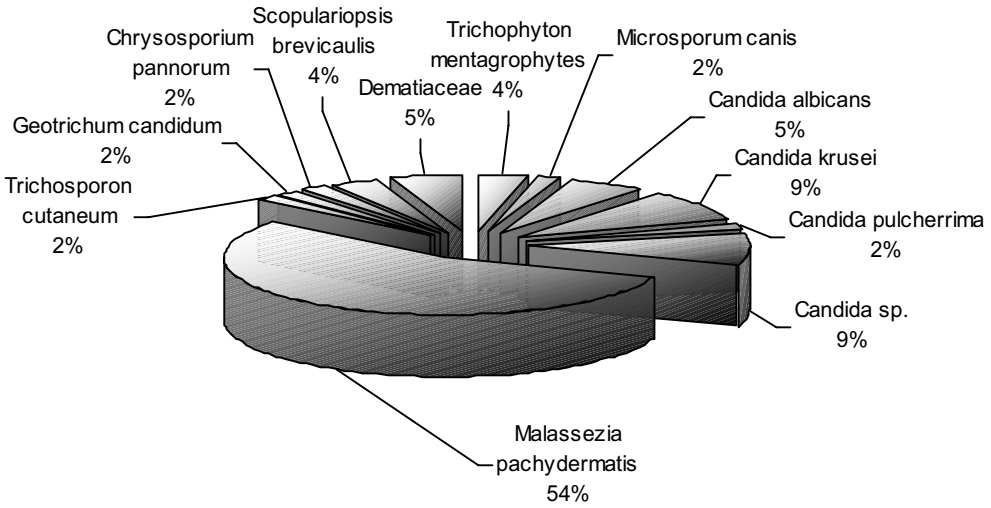

Fig. 1. Proportion of individual micromycetes species.

In 23 % of dogs affected by *Malassezia pachydermatis*, we observed a mixed infection in which also Staphylococcus sp. PLK+ (Fig. 3) was involved.

Candida sp.

The cultures of yeast-like micro-organisms that could not be identified because of their considerable contamination and did not belong to the basic four fermentation types were referred to as *Candida sp.* This took place in 5 cases and was involved in 1-year-old dog and 6-year-old bitch both of Shar-pei bread from the same breeding station. They were "mother and son". Additional cases were a 3-year-old French Bulldog bitch, 5-year-old Basset Hound and a 1-year-old mongrel. *Candida sp*. affected mostly young dogs.

Candida krusei (Castellani) Berkhout 1923

The agent was isolated from 5 samples. It was involved in dogs 1 to 4 years old and affected German Shepherds, Miniature short-haired Pinschers, Shar-peis, Bull Terriers and Maltese Dogs (Fig. 4)

Candida albicans (Robin) Berkhout 1923

This is a well known yeast-like micro-organism. It was isolated from 3 samples of dog skin. It was involved in 3, 2, and 1-year-old dogs. As to their breeds, 2 were Cocker Spaniels and one was Staffordshire Terrier (Fig. 5).

Trichophyton mentagrophytes (Robin) Blanchard 1896

At of 100 samples of skin scrapings and swabs from 100 dogs of both sexes, various breeds and age categories, exhibiting skin problems, *Trichophyton mentagrophytes* species was isolated from 2 samples. The first case was a 1-year-old Schnauzer and the second one a 3-year-old mongrel. In both cases the material was sampled with the suspicion of dermatophytes being involved. The dogs had foci on their heads and backs accompanied with intensive pruritus.

Microscopical findings in the material: chains of spores were observed inside the hair. In both cases the growth of strains was very rapid. On day 5, their length reached 1 to 1.5 cm on average.

Scopulariopsis brevicaulis (Sacc.) Bainier 1907

A 5-year old Golden Retriever and a 1-year-old Newfoundland bitch showed positivity to micromicete *Scopulariopsis brevicaulis*. Immediately after its isolation, we informed the owners of both dogs because the agent is highly contagious and is a relatively frequent cause of human contact onychomycosis which is resistant to all antimycotics. In coincidence with this agent we consider it very important to differentiate cultures of species *Chrysosporium pannorum* and *Scopulariopsis brevicaulis*. *Chrysosporium pannorum* is finer, its growth is less bulky,

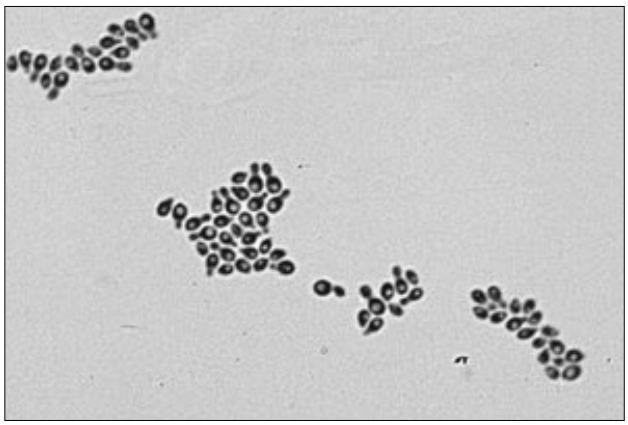

Fig. 2. *Malassezia pachydermatis* — microscopical diagnosis.

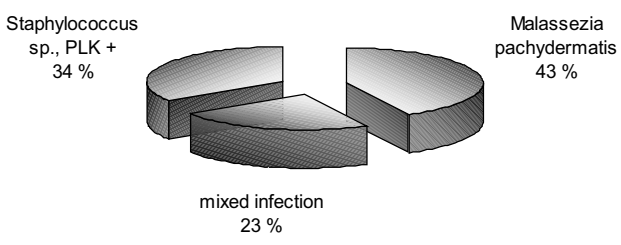

Fig. 3. Comparison of etiology of dermatopathies.

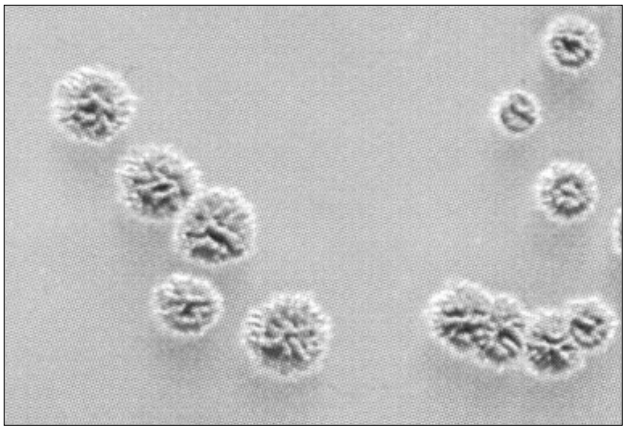

Fig. 4. *Candida krusei* – growth of the colonies (cultivated on Sabouraud agar).

forms abundant clusters in the preparation and conidio-carriers are finer and thinner.

The difference between conidia: Chrysosporium – oval, teardrop-shaped, Scopulariopsis – spherical, frequently spurred.

The base side of both conidia is cut up.

Microsporum canis Bodin 1902

The species *Microsporum canis* was isolated only in one case, from a skin scraping of a 7-year old Cocker Spaniel bitch, together with serious infection caused by

Candida pulcherrima (Linder) Windish

This relatively rare candidal species was isolated from skin of an 8-year old Dalmatian bitch. It is one of primitive yeast micro-organisms, capable only of slight fermentation of monosaccharides, glucose, maltose, and fructose. No exact literary data are available on their pathogenicity.

Trichosporon cutaneum (de Beurman, Gougerot et Vaucher) Ota 1926

This agent was isolated from a 3-year old Laika. Colonies were observed on agar plates as soon as after 24 hours. They were cream or white coloured, smooth, and later on frizzy and rough. It was the presence of blastospores that distinguished this agent from the morphologically similar genus *Geotrichum sp*.

Geotrichum candidum Link 1809

A 1-year-old mongrel bitch became the source of isolation of this yeast that occurs relatively rarely on skin. It produced fine fur-like white-coloured colonies on nutrient agar, spread widely and growed rapidly.

Chrysosporium pannorum (Link) Hughes

The agent was isolated from the skin of a 1-year-old pug bitch.

The macroscopic colonies had a cumulus-like appearance. They were white, slightly dusty and became light grey after some time. Some of them were convex and striated. The base was yellow and the pigment penetrated slightly into agar.

The macroscopical observation revealed sparse, irregularly branched conidiophores. Aleuriospores had one bluntly cut end formed at the ends of the branch. Microconidia had a typical teardrop shape.

Fig. 5. *Candida albicans* – growth of the colonies (cultivated on Sabouraud agar).

Out of the saprophytic fungi of the family of Dematiaceae, we isolated *Cladosporium sp.* and *Alternaria sp*.

Discussion

In general, the dermatological diseases constitute a serious anthropozoonotic problem in pet-keeping (Kozák et al, 1998). Ditrich, Rosický and Otčenášek (1990) described urban cycles of some mycotic species of zoonotic character. Hulinger et al (1999) described the situation in the USA.

Mycotic diseases in animals are either manifested by typical disease changes or persist asymptomatically without evident clinical signs. A too late recognition of the disease or too discrete symptoms of untreated animal mycosis can lead to the development of human infections (Brasch et al, 1998; Huovinen et al, 1998; Jury et al, 1999; Patel, 2000; Baranová and Martinásková, 2001; Chiller et al, 2002). Working and breeding relations between humans and animals can expose humans to the risk of infection. The most frequent carriers of zoophilous species are the domestic animals, particularly the long-haired species (dogs, cats, guinea pigs, hamsters, and others), but also stray animals. Mycosis can also be transmitted by contaminated materials (straw, feed, and others) or via soil. Children are the most endangered group because of their close emotive relationship with animals.

Microsporia are generally considered to be the agents of most contagious mycotic diseases. *Microsporum canis* is the most frequent microsporum agent in western Europe but also on other continents. According to Otčenášek, Komárek and Dvořák (1974) *Microsporum canis* was responsible in the past for the highest proportion of dermatophytoses of dogs and cats under our geographical conditions. This was confirmed later by Danilla and Antošovský (1983), Danilla and Volleková (1986), Fábiková et al (1995), and Hošek (1999). According to these authors, 3–5 % of dermatological patients are affected by dermatophytoses while the incidence in cats is still higher (Hošek, 1999). In addition to the mentioned species, the latter author mentioned also *Mi-*

crosporum gypseum as an occasional seasonal agent, particularly during summer months. *Microsporum gypseum* is a geophyllic species.

In our study *Microsporum gypseum* was isolated only in one case. The animal affected was a 7-year old Cocker Spaniel bitch.

The species *Trychophyton mentagrophytes* was identified by several authors as the second most frequent agent of dermatophytoses in dogs and cats. Georg (1962) cited by Komárek and Wurst (1989), Danilla and Antošovský (1983), Danilla and Volleková (1983), Morganti et al (1980) examined dogs living in urban environment. Out of 300 examined samples, the species *Trychophyton mentagrophytes* was isolated 8 times. In 1969–1989 Vokoun and Kučera (1991) conducted an extensive study on dermatophytes in cats and dogs in the urban environment and pointed out that the clinical picture was diverse. The results of this twenty-year-study showed predominance of findings of the species *Trichophyton mentagrophytes*. Out of 836 samples taken from dogs, this species was isolated in 48 cases.

The incidence of the species *Trichophyton mentagrophytes* in humans was described by Baranová and Martinásková (2001), and Czaika et al (1998). Huovinen et al (1998) recorded onychomycosis in a man caused by *Trichophyton equi*num transferred by contact with a riding horse.

Yeast micro-organisms of genera *Candida sp*. and *Trichosporon sp*. have been referred to as secondary pathogens which can infect patients with immunologic disorders, metabolic diseases and those who were treated with corticosteroids, hormonal preparations and broad-spectrum antibiotics. Infections may occur in bitches even under physiological conditions during oestrus and gravidity. The development of candidosis depends also on the complex of relations between the pathogenicity of an microorganism and the protective mechanisms of animals (Šimaljaková, 1997). In our study *Candida* species were found also in young dogs free of immunologic disorders. Species of the genus Candida are frequent agents in different diseases of man (candidosis of respiratory and urinary tract, nervous system, eyes, skin, mucosae and others) (Dorko et al, 2000, 2001d, 2002a). Jones and Russel (1974) and later Dorko et al (2001 a,b,c; 2002 b,c,d,e) were the first to demonstrate the significance of yeast organisms in the development of oral candidosis.

The occurrence of *Malassezia pachydermatis* in the set of our patients corresponded with the reports of other authors published at home and abroad (Gueho et al, 1987; Kocková, Ladzianska and Bučko, 1987; Scott and Miller, 1989; Černá, 1994; Rybníček, 1997; Svoboda et al, 1998; Beladičová, 2000). Fábiková et al (1995) examined 634 patients with symptoms of skin diseases, including 502 dogs. Their results are in agreement with our findings as the most frequently found agents included *Malassezia pachydermatis* and yeast microorganisms of the genus of *Candida*. Medical literature contains data about occurrence, course of the disease, laboratory diagnostics, treatment, and trials to prove the pathogenicity of this very frequent species (Baxter, 1976; Fernandez, 1987; Jarvis et al, 1998). Carlotti (2001) published papers concerning the diagnosis of *Malassezia dermatitis.* It deserves attention particularly due to the fact that the knowledge about its epizootiology is scarce. When examining the cultures, we became interested in their morphological diversity that was probably caused by external factors. The budding observed in our cultures occurred at the wide base in agreement with Nishimura et al (1991), Černá (1994), Huang et al (1993), and others. In relation to isolation of this species from samples of human organs it is necessary to indicate that system diseases can possibly develop not only in animals. Gue ho et al (1987) isolated the agent from 15 samples taken from human organs. Chang et al (1998) (Beladičová, 2000) described an infection in babies at the neonatal unit that had been transferred by an attendant who kept at home 3 dogs infected with *Malassezia pachydermatis*. The identical species was isolated from samples from all these little patients. Eight neonates had positive haemocultures, 2 had urogenital tract infection, one suffered from meningitis and 4 were asymptomatic but their weight gain was lower compared to other healthy children. Ali-Shtayeh et al (1998), Aste et al (1997), and Baranová (2000), Baranová and Martinásková, (2001) reported similar cases in children at school age.

From the aspect of clinical practice and subsequent therapy we consider the relatively frequent joint occurrence of agents *Malassezia pachydermatis* and Staphylococcus sp. PLK+ to be important. The study by Pomorski, Blimke and Pomorska (2001) confirms this opinion.

Our effort to find information about pathogenicity of this agent was in literature unsuccessful. Breirová et al (1991) and Černá (1994) tried to prove the production of extracellular glycoproteins as the potential indicators of pathogenicity. Černá (1994) examined 27 positive samples and proved that 16 strains produced extracellular proteins.

It should be noted that the nomenclature of this species underwent a complicated development. Gueho and Meyer (1989) stated that *Pitysporum ovale* and *Pitysporum orbiculare* are only synonyms of the human species *Malassezia furfur* and this is the presently preferred opinion.

Trichosporum cutaneum is considered to be an agent of both skin and systemic diseases. Volleková (1998) described the role of this species and of other similar agents in etiology of mycoses. The disease caused by *Trichosporum cutaneum* is referred to as trichosporosis. Its isolation under laboratory conditions is not rare in case of mixed infections. Despite that Beladičová (2000) recorded only one case caused by this agent in the whole set of patients examined.

Fassatiová (1999) pointed out that the species *Chrysosporium pannorum* is variable. The isolation of the species *Chrysosporium keratinophilum* was reported by Otčenášek, Dvořák (1978), Gaur and Ligchtwardt (1980). Zelenková and Jautová (2002) reported an interesting finding concerning the species *Chrysosporium pannorum* in dog and its owner. The disease was first diagnosed in the owner who underwent an unsuccessful long-term treatment at the skin department. Later it was found out incidentally, that the patient's dog of Chow-Chow breed had similar skin changes. *Chrysosporium pannorum* was isolated from both the dog and its owner. Both were subjected to pulse antimycotic treatment which was successful in both of them.

In our study we isolated *Chrysosporium pannorum* only in one case (pug) and neither the owner nor other members of the family were affected.

Geotrichum candidum was isolated rarely from the skin samples. Out of 100 samples examined in our study only one was positive for this fungi. It involved a 1-year old mongrel bitch. This microscopic fungi represents a transition between yeasts and filamentous fungi and is frequently isolated from milk products. It has been reported that this fungi is capable of degrading aluminium. It impaired compact discs in Germany, Spain, USA, Mexico and Taiwan. Information about the isolation of the species *Geotrichum candidum* from an abscess was reported by Morenz (1983). Fassatiová (1999) presented this species as a saprophytic fungi that under certain conditions can become pathogenic in mouth cavity or respiratory tract.

Scopulariopsis brevicaulis is found relatively frequently in dogs, cats, and large farm animals, particularly horses (Beladičová, 2000). Despite that, out of 100 samples examined in our study, only 2 were positive for this agent. This fungi shows an affinity to claws, nails, and hoofs. It is possible that our findings were affected by the fact that did not take samples from claws.

According to many authors, the isolation of this species is associated with problematic treatment. It causes frequently onychomycoses that are resistant to all antimycotics (Skořepová, 1991; Vosmik, 1995).

Saprophytic fungi of the family of *Dematiaceae* were included among our mycological findings only due to the fact that we wanted to determine their incidence in skin scrapings and skin swabs of dogs.

Two genera were isolated, namely *Alternaria sp*. and *Cladosporium sp*. We considered it important in relation to the problems with the diagnosis of dermatophytes outside the laboratories. At the present, the so-called „dermatophyte test media" (DTM) are used for the typing of micromycetes at clinics. The medium contains a pH indicator. The growth of dermatophytes results in an immediate change in pH of the medium due to the metabolism of proteins that distinguishes them from saprophytic microscopic fungi that consume first sugars and cause no change in pH. The colour of the medium changes from yellow to red. The change in colour of the medium before or parallel with the growth of a white non-pigmented colony of microscopical fungi is considered a positive finding. Other findings should be considered negative. According to our experience, the veterinarians working at clinics who use the DMT media quite frequently refer to microscopical fungi of the family of *Dematiaceae* also as dermatophytes.

Microscopical fungi of the family of *Dematiaceae* produce colonies with dark brown, dark red, or even black base. The genus *Cladosporum* has a greenish-black base and later on produces dark purple pigment within the agar. This is a significant feature of this family. Particularly disputable are the colonies of the genus of *Alternaria* that are white at the beginning of their growth and are frequently mistaken for dermatophytes.

Our microscopical examination showed the presence of spores typical of these saprophytic fungi that cannot be mistaken for any other spores. Out of 100 examined samples, 3 were positive for microscopical fungi of the family of *Dematiaceae*.

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